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COMMENTS ON A PLASTICS INDUSTRY NEUROTOXICITY IN RELATIONSHIP TO METHYBUTYL KETONE

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PAPER NO. 7

COMMENTS ON A PLASTICS INDUSTRY NEUROTOXICITY IN RELATIONSHIP TO METHYLBUTYL KETONE

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INTRODUCTION

Recently a number of workers involved in a local plastics coating manufacturing plant were afflicted with peripheral neuropathies of varying degrees of severity. The clinical results of electrodiagnostic (EMG) screening of 1157 workers from this plant showed 965 had no abnormalities; 72 within normal limits; 24 with no definite abnormalities; 28 with suspected abnormalities; and 68 with definite signs, symptoms and electrodiagnostic findings of peripheral neuropathy (Mendell, 1974; Allen, 1974; Billmaier, 1974). A thorough epidemiological study revealed that the recent introduction of methylbutyl ketone (MBK) as a solvent in the dye and color printing processes might be linked to the neuropathy. This was suspected especially since plastics industries throughout the country had reported no known illness of this sort and processes were similar except that no other plant used methylbutyl ketone (Billmaier, 1974). It was inferred that methylbutyl ketone must be related to, or indeed the cause of, the peripheral neuropathy.

Analysis of air samples in the print shop area showed variable but consistently high ketone concentrations (methylethyl ketone, approximately 10-40 ppm). In addition to ketones, low levels of hexane, diacetyl, aromatic hydrocarbons and traces of other solvents were detected (Billmaier, 1974).

A search of the toxicology literature gave only a paucity of data available on MBK toxicity and essentially no studies on the effects of chronic exposure (Browning, 1965).

In response to the nature and urgency of this industrial toxicity and in view of the unknown agent(s) involved: a cooperative venture involving dozens of investigators at The Ohio State University emerged in an attempt to determine the cause of this neuropathy. This report describes our approach to the evaluation of methylethyl ketone and methylbutyl ketone as possible causative agents of the peripheral neuropathy.

METHODS

Toxicology Testing

Our initial efforts involved an examination of workers blood, urine, hair and nails for any contents which might lead to the identification of any known or suspected neurotoxin. A drastic limitation of this approach was that these biological samples were obtained from workers that had been away from the work environment for 3-6 weeks or more. Other samples were obtained from workers on the job only after MBK had been removed from the process. In any case, efforts were made to determine blood and urinary heavy metals, thiocyanates, ketones and ketone metabolites, total urinary glucuronides, thiamine, serum and RBC cholinesterases and plasma CPK. Analysis of heavy metals, lead, zinc, cadmium and thallium were done by atomic absorption spectrometry utilizing carbon rod atomization; mercury by flameless AA; arsenic by colorimetry. Cholinesterases were determined by the isotope method of Siakotos (1969). Other assays were performed according to currently acceptable standard methods.

Exposure of Animals

The purpose of these studies was to establish an animal model of peripheral neuropathy in order to demonstrate the etiologic relationship of a volatile solvent to this disease. We felt that the inhalational route was the most likely manner in which workers were exposed. We, therefore, utilized the inhalational route, exposing animals in a modified germ-free apparatus fitted for flow-through concentrations of vapors carried via a continuous air flow. Chambers were monitored to maintain O_2 at 21%, CO_2 at 0.1-0.2%, relative humidity at 30-50%, temperature 24 C. Concentrations of ketone vapors were determined by GLC and agreed with calculated volumes of liquid required to achieve the appropriate levels.

In addition, food and water were available in the chamber, consumption of food and water and body weight were recorded throughout the studies. Exposure to ketone vapors was considered at tolerable levels only if there was no diminution in food and water intake. If these were decreased, then ketone concentrations were lowered. Pair fed controls were subjected to similar conditions in the absence of the ketone vapors.

It was not readily predictable whether or not a peripheral neuropathy would be produced by ketone vapors or even occur in an animal model. In the preliminary studies we used several species which included chickens, mice, rats and cats. We found that all species except the mouse exhibited a typical clinical peripheral neuropathy. The chicken was the most sensitive species showing severe neuromuscular impairment at 100 ppm MBK after three weeks of continuous exposure. Rats were more manageable and motor nerve preparations were examined for ultrastructural alterations after solvent exposure. Cats exhibited peripheral neuropathies and were especially suitable for electrodiagnostic evaluations of neuromuscular function.

In all systems, exposure was continuous, 24 hours a day, seven days a week, but with the following interruptions: a 15 minute break each day for all chambers for measuring and replenishing feed and water; a 24 hour break once a week for the cats only, when they were removed for electromyogram studies. Also, at one to two week intervals, chickens, rats and mice were removed from the chambers for 30-60 minute intervals for obtaining body weights and blood samples.

Electromyographic Studies

Electromyographic studies and ulnar nerve conduction measurements were carried out on all exposed cats and their controls on a weekly basis according to methods described by Chrisman (1972).

In some exposure experiments, cats that exhibited early and marked neuropathies were removed from MBK or MEK-MBK vapors and were handled as controls for observation for recovery processes.

Histology

The sciatic nerves of animals exposed to MBK or MEK-MBK and their appropriate controls were fixed in situ with 3% glutaraldehyde in 0.1M phosphate buffer pH 7.5. The nerves were postfixed with 1% osmium tetroxide and embedded in Spurr low-viscosity media. Sections were studied by light microscopy. Thin sections of portions of the nerve were viewed with a Hitachi Hu 12 electron microscope. Other portions of the nerve were prepared for nerve fiber teasing (Dyck, 1968).

RESULTS AND DISCUSSION

Toxicology Testing

The results of heavy metal analyses in urine and blood of the workers are summarized in Table 1.

TABLE 1. METAL ANALYSIS

Metal	Results	Assay Sensitivity (ppb)
	Blood (91 workers)	
Lead	within normal limits	5.0
Zinc	within normal limits	5.0
Mercury	within normal limits	1.0
	Urine (91 workers)	
Arsenic	within normal limits	10.0
Cadmium	within normal limits	0.1
Mercury	within normal limits	1.0
Thallium	within normal limits	1.0
Zinc	within normal limits	5.0

All values were found to be within normal limits, or not detectable. Analyses for other nonmetallic agents (Table 2) were also within normal limits, the few elevated urinary thiocyanates were not considered significant and not pursued.

TABLE 2. NONMETALLIC AGENTS

Number of Samples	Test	Results
12	Acetone bodies (blood)	within normal limits
120	Acetone bodies (urine)	within normal limits
99	Halogenated Hydrocarbons (urine)	within normal limits
85	Thiocyanate (urine)	six elevated values

Additional analyses were carried out on the technical grade solvents MBK, MEK and the recovered recycled MEK-MBK. Gas chromatographic analysis using an alkaline flame ionization detector did not indicate any phosphorus containing compounds (e.g., TOCP and other organophosphates). Infrared

		RBC µmoles/ mg/ hr	SERUM mmoles/ ml/ hr
A.	Normals (workers) N = 10	1.74 ± 0.57	0.45 ± 0.15
B.	Samples (print shop workers) N = 10	1.42 ± 0 48 (a) $p > 0.5$ (b) $0.2 > p > 0.1$	0.37 ± 0.13 (a) $p > 0.5$ (b) $0.4 > p > 0.2$
C.	Normals (hospital patients with normal liver function) N = 14	2.03 ± 0.14	0.25 ± 0.02
D.	Samples (workers -- initial) N = 96	1.37 ± 0.06 (c) $p < 0.001$ (d) $0.2 < p > 0.1$	0.64 ± 0.02 (c) $p < 0.001$ (d) $0.05 > p > 0.02$

Cholinesterase values represent the mean ± SEM
Significance levels, p values, by Student's Test

(a) Comparison was made between groups A and B
(b) Comparison was made between groups B and C
(c) Comparison was made between groups C and D
(d) Comparison was made between groups A and D

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analysis of recycled solvent after fractional distillation failed to demonstrate any nitrile containing contaminant. In the search for any possible contaminants occurring in the workers environment, we were limited to the study of unused solvents MBK and MEK exclusively. Unfortunately, we could not obtain any material actually used in the printing process which would have included a mixture of solvents with various components of the colors, dyes, resins, adhesives, plasticizers, detergents, color stabilizers, antimicrobials and fire retardants. The advantage of examining the solvent mixture would be to include the possibility not only that one of the process chemicals itself was potentially toxic, but that a toxic substance might be formed by reaction between chemicals under the temperature, humidity and conditions of the printing processes. Also, MBK was no longer used in the plant and the crude mixture would lack components (if any) extractable or soluble in MBK.

Cholinesterase determinations of about 100 workers who were judged to have subclinical manifestations of neuropathy showed a significant elevation of serum butyryl cholinesterase and an equally significant fall in erythrocyte acetyl cholinesterase (Table 3, Group D).

In employees judged no longer EMG-positive and in ten employees not directly involved in the print shop operation, there was no significant difference in their serum and erythrocyte cholinesterase levels and all appeared to be within normal range (Table 3, Group A vs B). There is some indication that in at least a few cases where symptoms developed later, these individuals had unusually high plasma butyryl cholinesterase and low erythrocyte cholinesterase.

TABLE 3. CHOLINESTERASE ACTIVITY

	Control	MEK	CT Value*
Mouse			
RBC	0.44		
	p = 0.02**	0.11	125
	p = 0.3	0.24	43,200
Serum	1.6		
	p < 0.001	4.1	125
	p = 0.003	2.5	43,200
Rat			
RBC	0.40		
	p = 0.02	0.14	125
	p = 0.03	0.19	43,200
Serum	0.15		
	p = 0.008	0.30	125
	p < 0.001	0.27	43,200
Chicken			
RBC	0.013		
	p = 0.008	0.025	32,000
Serum	0.57		
	p < 0.001	1.3	32,000

[a] RBC acetylcholinesterase activity expressed as μmoles/ mg protein/ hr.

*CT Value = Concentration (ppm) x time (days).

**p < 0.01 is statistically significant.

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The influence of chronic exposure of mice, rats, and chickens to MEK on cholinesterase activity is presented in Table 4. It is important to note that there is in all cases a statistically significant elevation in plasma cholinesterase levels following chronic exposure. In some cases, MEK produced greater increases than MBK (data not shown) but this may be due in part at least to the much greater CT (concentration x time) experience with MEK. The rodents exhibit a significant decrease in erythrocyte cholinesterase which is not shared by the chicken. This may reflect the difference between avian erythrocytes which are nucleated, and hence capable of protein resynthesis, and rodent erythrocytes which are nonnucleated.

Determinations of serum CPK in workers as well as animals exposed to solvent vapors were in the normal range.

TABLE 4. EFFECT OF MEK EXPOSURE ON CHOLINESTERASE ACTIVITY[a]

Exposure of Animals

After preliminary range finding experiments with solvents and species, the following exposure pattern was employed.

In separate m^3 chambers, animals were exposed to vapors of MBK, MEK or a mixture of MEK-MBK vapors (10:1). In one chamber four cats, four rats, and five mice were exposed to MBK starting at 75 ppm and doubling at approximately weekly intervals until 600 ppm was reached. Another similar chamber exposed animals to a combination of MEK and MBK 10:1, starting at 500:50 ppm of each, doubling these levels to a maximum of 2000:200 by the third week. Another chamber with chickens alone had initial levels of 50 ppm MBK increasing to 200 ppm by the third week. In each case the peak level was to be maintained for at least six weeks, providing severe adverse reactions did not occur in the animals. Food and water intakes and/or body weights falling more than 10-25% below controls would be taken as a general toxic response likely to confuse the development of peripheral neuropathy, and would necessitate a reduction in solvent concentration.

Choice of solvent concentration and continuous exposure was dictated in part by the desire to obtain a rapid response but was modified by the appreciation that overwhelming toxicity would likely obliterate the more subtle aspects such as nerve conduction and EMG changes and possibly even gross signs of paralysis. Therefore, a relatively low initial level and a stepwise increase in solvent concentration was adopted with continuous observation of animal performance along the way. Peak levels to be reached were established in preliminary trials in which rats and mice would tolerate 600 ppm MBK; cats might, and chickens would not. Also preliminary trials showed that 2000 ppm MEK was tolerable for rats and mice. It seemed prudent to increase chamber concentrations in a cautious stepwise manner. The ratio 10:1 for MEK:MBK was suggested as being representative of the working conditions in the plant.

The chronology of effects observed by continuous exposure of 4 species of animals to MBK vapors is summarized in Figure 1. The data indicate that chickens were the most sensitive species. MBK (200 ppm) caused some decrease in food and water intake in the first 3-5 weeks; the sixth week MBK was reduced to 100 ppm. In the fifth week, chickens showed signs of leg weakness, manifested initially by reduced activity and unsteady gait, soon thereafter (1-2 days) the chickens could not stand on their legs at all. From week 5-12, chickens were sacrificed for histology as soon as they exhibited the progression of mild to severe symptoms. The restricted-fed chickens (controls) had similar weight losses but no leg weakness.

Cats, rats and mice tolerated higher levels of MBK than chickens but, as seen in Figure 1, after several days at 600 ppm, a depression in food and water intake prompted a reduction to 500 then 400 ppm MBK. The first sign of neuropathy, positive waves in electromyography (EMG), also occurred

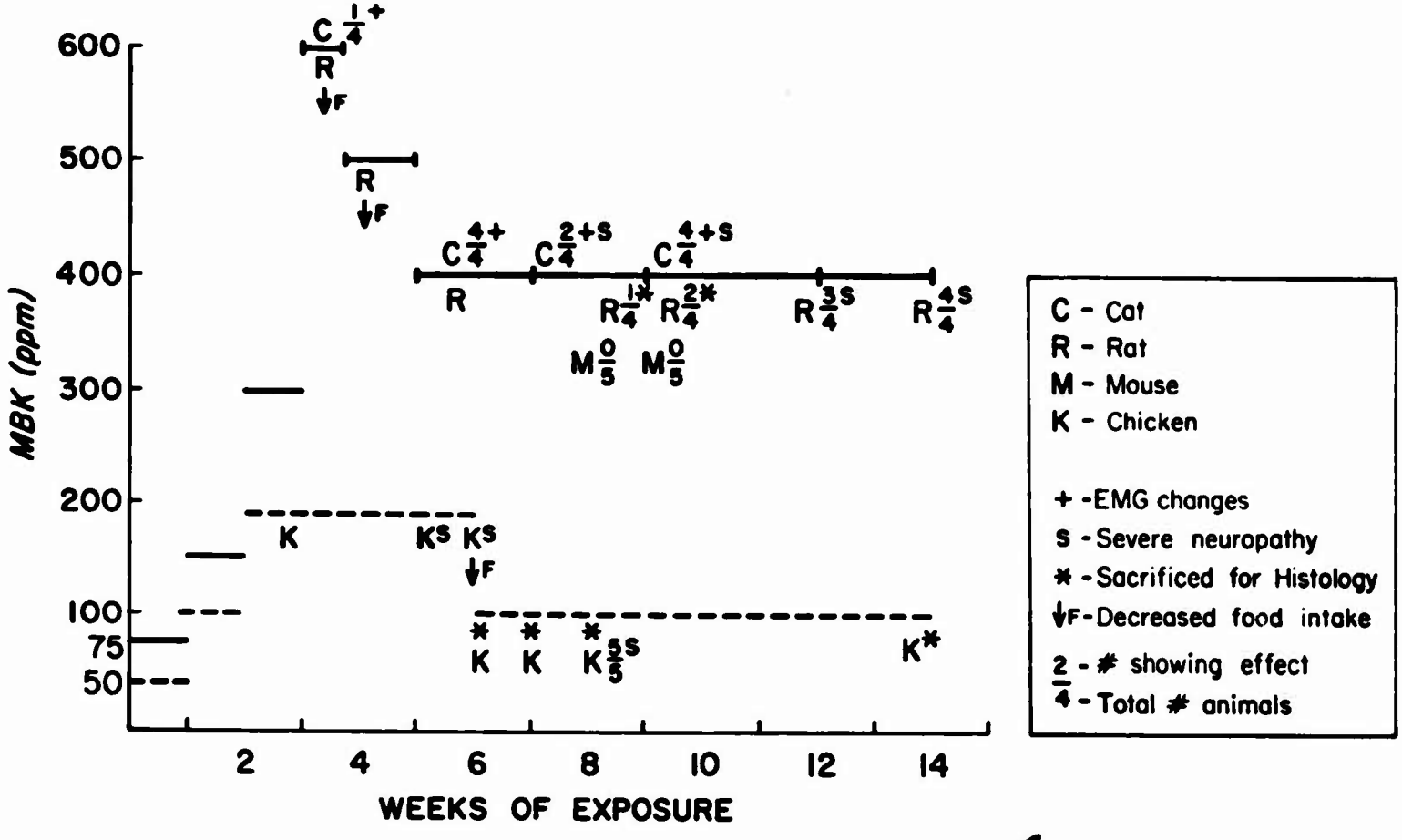

MBK (ppm)
600
500
400
300
200
100
75
50
2
4
6
8
10
12
14
WEEKS OF EXPOSURE
C - Cat
R - Rat
M - Mouse
K - Chicken
+ -EMG changes
S - Severe neuropathy
* - Sacrificed for Histology
↓F - Decreased food intake
2/4 - # showing effect / Total # animals

FIG 8

during 600 ppm exposure. Chickens developed severe paralysis in a minimum of 5 weeks in MBK 100-200 ppm, whereas cats showed leg weakness in this same time interval at 400-500 ppm MBK. Rats were more resistant than cats to MBK, requiring exposures of about 11-12 weeks at 400 ppm for the first signs of leg weakness to appear. Mice were exposed to 400 ppm MBK for 9 weeks and did not show any evidence of leg weakness. Mice gained weight comparable to controls.

Figure 1. Effect of MBK exposure on various species.

Other experiments were conducted with animals exposed to vapors of MEK alone, or mixtures of MEK-MBK. The results are summarized in Table 5 depicting the solvent concentrations, time to occurrence of paralysis for each species, and the variation in response. Noteworthy is the absence of any paralysis in the mouse with all solvents tested. Also, exposures to MEK alone did not cause paralysis in any of the animals (exposure was 7-9 weeks, 1500 ppm).

TABLE 5. TIME TO OCCURRENCE OF CLINICAL PARALYSIS

	Chicken	Cat	Rat	Mice
MBK (ppm)	200	400	400	400
	4 weeks	5-8 weeks	12 weeks	none
MEK (ppm)	1500	1500	1500	1500
	none	none	none	none
MEK-MBK (ppm)	1500-150	1500-150	1500-150	1500-150
	4 weeks	5-8 weeks	6 weeks	none

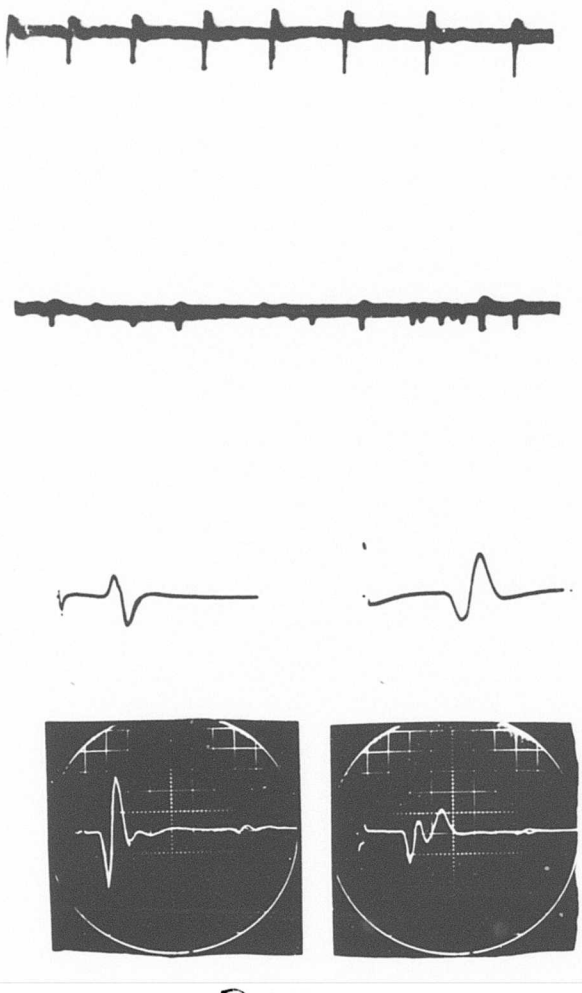
Interaction Between MEK and MBK

In rats exposed to a mixture of vapors of MEK-MBK, 1500:150 ppm, paralytic signs were observed in about 6 weeks; whereas in 400 ppm MBK alone 11 weeks elapsed before leg weakness appeared. Cats were somewhat wobbly in MEK-MBK 1500:150 ppm, as if partly anesthetized. While this unsteadiness did not appear equivalent to the leg weakness and to paralysis seen in MBK alone, it may be difficult to separate the two conditions in their earlier phases. Food intake was also more depressed in the MEK-MBK combination than with MBK alone. In addition, the MEK-MBK cats showed a prolonged duration of sleep after pentobarbital anesthesia (given for the electrodiagnostic tests). Mice were equally little affected by either MBK alone or MEK plus MBK. The data obtained from rats and cats exposed to MEK-MBK suggest that MBK is more toxic in combination with MEK.

Electromyographic Studies

Each cat used in these experiments was tested by electromyography at weekly intervals prior to and during exposure to the solvent vapors. Recording of the electrical activity of the muscle at rest and during insertion of the electrode was obtained in anesthetized cats (pentobarbital 30 mg/kg). Typical electrodiagnostic data is presented in Figure 2. The top row tracings show positive waves which are the first sign of muscle irritability; these were seen as early as 4-6 weeks in all the cats exposed to MBK (see Figure 1). Fibrillation potentials along with positive waves (Row 2) indicate severe disease of the motor unit.

After 7-9 weeks, all the cats showed ulnar nerve conduction velocities slowed to about 50% of their 115 m/sec control values (Row 3 vs 4). The EMG findings occurred in all muscles tested, with the more marked changes noted distally. Cats exposed to both MEK-MBK exhibited electromyographic changes similar to those observed with MBK.



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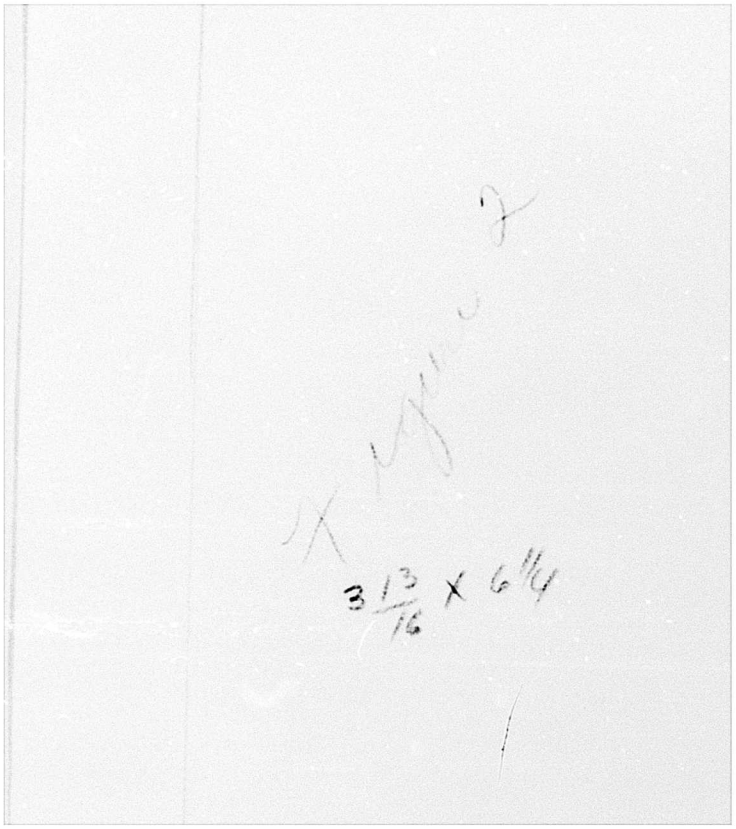

Figure 2. Electromyography patterns after MBK exposure.

Top Row: Train of positive waves in the anterior tibialis muscle after 6 weeks of exposure to MBK in the cat.

2nd Row: Fibrillation potentials in the triceps after 9 weeks of MBK exposure in the cat.

3rd Row: Prolonged latency between stimulus and recorded muscle action potential from ulnar nerve stimulation (wrist to food pad on the left and elbow to food pad on the right).

4th Row: Control recording showing stimulus artifact and recorded foot pad muscle action potential from stimulation of ulnar nerve (wrist to food pad on the left and elbow to foot pad on the right).

Histology

During exposure to MBK or MEK-MBK, some rats, cats, and chickens exhibiting neuropathies of varying severity were sacrificed (see Figure 1) for histologic studies. Exposure to MBK or MEK-MBK, but not MEK alone, caused similar pathological changes in nerve preparations of all three species. A composite of results of the histologic examination after MBK is shown in Figure 3. The salient findings were: axonal swelling, oftentimes para Nodal (Figure 3a, 3b); denudation of myelin and thinning of myelin (3c); a greatly increased number of neurofilaments with fewer microtubules (3c). None of these histologic alterations occurred after MEK alone.

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Figure 3. Effects of MBK on rat sciatic nerve preparations.

a Teased nerve fiber showing areas of axonal swelling (arrows). Node of Ranvier seen at the arrowhead. X 100

b Thick section of epoxy resin embedded nerve showing swollen axon and denudation of myelin (arrow). The normal size of axon can be seen at Node of Ranvier (arrowhead). X 500

c Electron micrograph through area of axonal swelling showing thinning of of myelin sheath (M) and decreased number of microtubules (MT) compared to neurofilaments (NF) which are increased. X 43,200

Comment

This study demonstrated that the continuous exposure of animals to MBK can produce leg weakness which progresses to a paralytic neuropathy in chickens, cats and rats, but not mice. Many clinical features of the peripheral neuropathy in the workers were also demonstrable in the animals, for example, muscle weakness, alterations in electromyogram, RBC and serum cholinesterase changes. The histologic changes in animals remain unknown for man since no nerve biopsies were obtained.

The results obtained do not necessarily prove the MBK was the causative agent in the human neuropathies. It is not inconceivable, and indeed, quite possible, that other substances initially present or formed in the process and extractable by MBK are the primary toxic agents. What our results demonstrate quite clearly is that MBK under the conditions described can produce a condition similar in many respects to the human neuropathy.

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